

**A**

**pT-1 serine analogues abolish Plk1 PBD : peptide binding in solution**

| Peptide name        | Peptide sequence                         | $K_d$       |
|---------------------|--|-------------|
| PoloBoxtide-optimal | MAGPMQ-S-pT-P-LNGAKK<br>(SEQ ID NO: 3)   | 280 ± 27 nM |
| PoloBoxtide-7A      | MAGPMQ-A-pT-P-LNGAYKK<br>(SEQ ID NO: 68) | N.D.B.      |
| PoloBoxtide-7G      | MAGPMQ-G-pT-P-LNGAYKK<br>(SEQ ID NO: 69) | N.D.B.      |
| PoloBoxtide-7C      | MAGPMQ-C-pT-P-LNGAYKK<br>(SEQ ID NO: 70) | N.D.B.      |
| PoloBoxtide-7T      | MAGPMQ-T-pT-P-LNGAYKK<br>(SEQ ID NO: 71) | N.D.B.      |

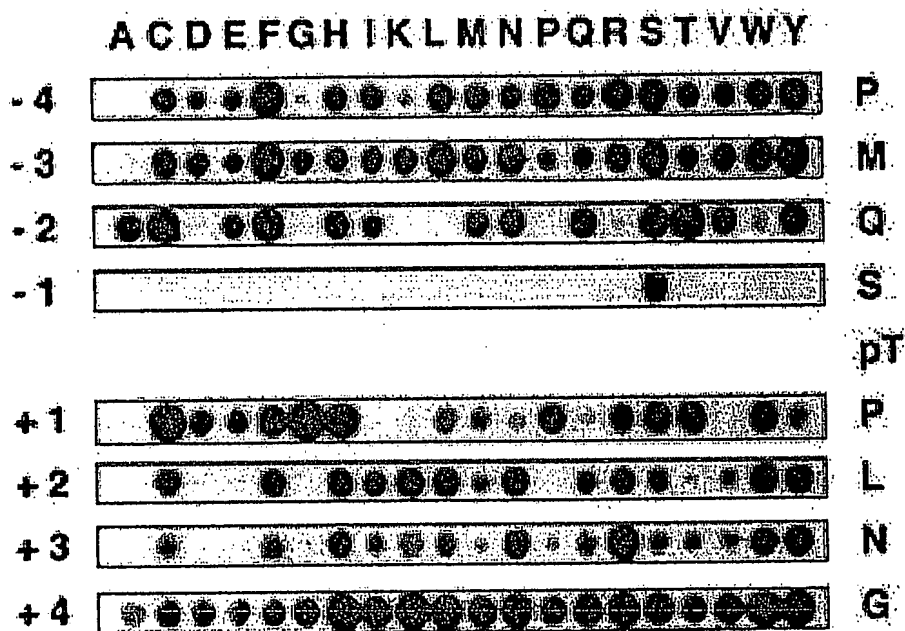
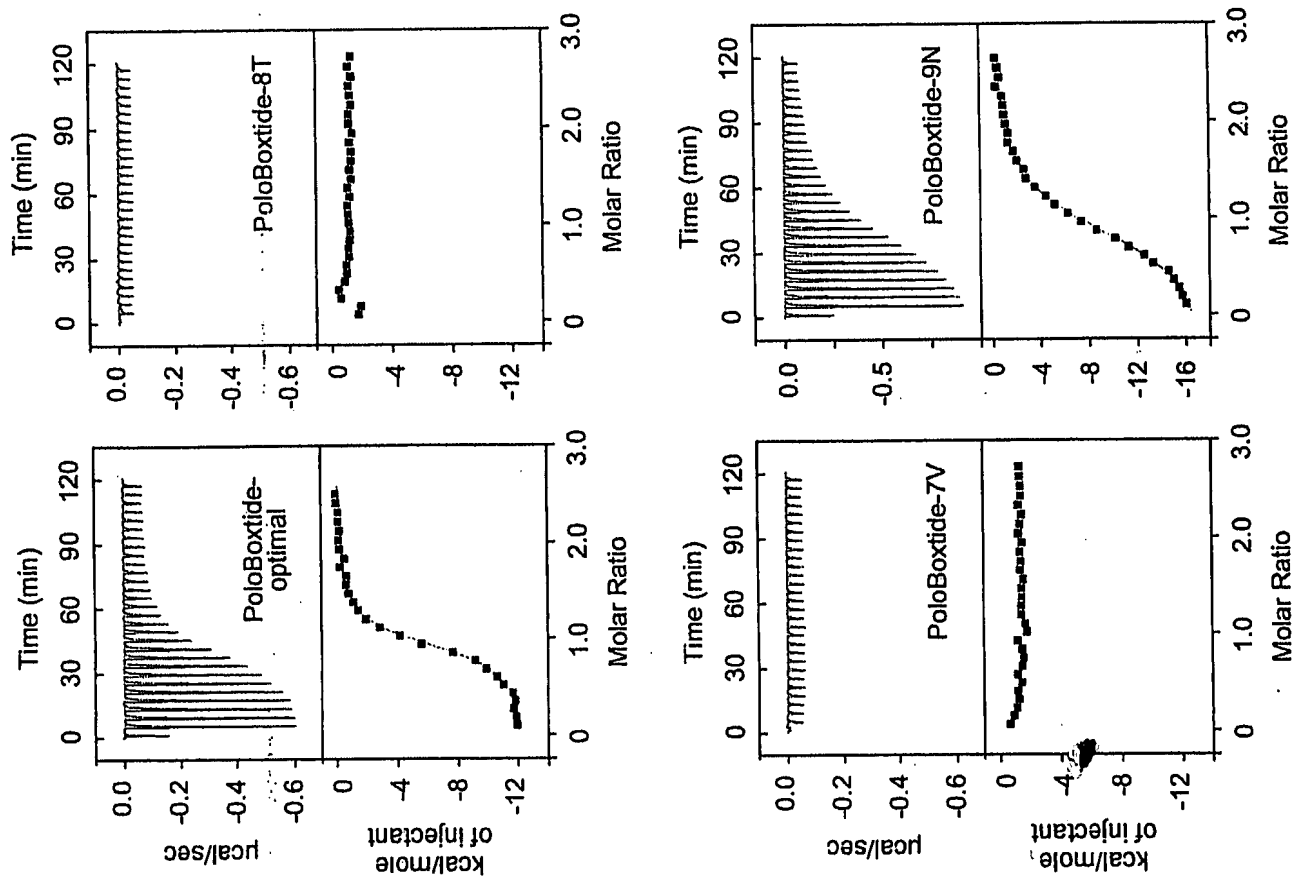
**B**

Figure 9



| Peptide binding affinities for the Plk1 Polo Box Domain |   |                 |  |
|---|---|-----------------|--|
| Peptide name  | Peptide sequence                                  | $K_d$           |  |
| PoloBoxide-optimal                                      | MAGPMQ-S-pT-P-LNGAKK<br>(SEQ ID NO: 3)            | 280 $\pm$ 27 nM |  |
| Effect of pT  |   |                 |  |
| PoloBoxide-8T   | MAGPMQ-S- <b>T</b> -P-LNGAKK<br>(SEQ ID NO: 34)   | N.D.B.          |  |
| PoloBoxide-8pS  | MAGPMQ-S- <b>pS</b> -P-LNGAYKK<br>(SEQ ID NO: 65) | 2.1 $\mu$ M     |  |
| PoloBoxide-8pY  | MAGPMQ-S- <b>pY</b> -P-LNGAYKK<br>(SEQ ID NO: 66) | N.D.B.          |  |
| Effect of serine at pT-1 position                       |   |                 |  |
| PoloBoxide-7V   | MAGPMQ- <b>V</b> -pT-P-LNGAKK<br>(SEQ ID NO: 67)  | N.D.B.          |  |
| PoloBoxide-7A   | MAGPMQ- <b>A</b> -pT-P-LNGAYKK<br>(SEQ ID NO: 68) | N.D.B.          |  |
| PoloBoxide-7G   | MAGPMQ- <b>G</b> -pT-P-LNGAYKK<br>(SEQ ID NO: 69) | N.D.B.          |  |
| PoloBoxide-7C   | MAGPMQ- <b>C</b> -pT-P-LNGAYKK<br>(SEQ ID NO: 70) | N.D.B.          |  |
| PoloBoxide-7T   | MAGPMQ- <b>T</b> -pT-P-LNGAYKK<br>(SEQ ID NO: 71) | N.D.B.          |  |
| Effect of proline at pT+1 position                      |   |                 |  |
| PoloBoxide-9N   | MAGPMQ-S-pT- <b>N</b> -LNGAKK<br>(SEQ ID NO: 72)  | 1.5 $\mu$ M     |  |

Figure 4